

**In the Specification:**

Please amend the specification as shown:

Please delete paragraph [0045] and replace it with the following paragraph:

[0045] Fig. 4 shows kinetic data in the form of a double reciprocal plot ( $1/V$  plotted as a function of  $1/S$ ) obtained with a protein kinase A for several concentrations of ATP (50, 10, 3 and 2  $\mu$ M) in the absence (lowest trace) or presence of the inhibitor staurosporine (5 nM, middle trace) or the PKA-specific peptide inhibitor TYADFIASGRTGRRNAI (SEQ ID NO: 20) (20 nM, highest trace).

Please delete paragraph [0065] and replace it with the following paragraph:

[0065] Protein kinase recognition moieties having desired specificities for particular kinases and/or kinase families can also be designed, for example, using the methods and/or exemplary sequences described in Brinkworth et al., Proc. Natl. Acad. Sci. USA 100(1):74-79 (2003).

**TABLE 1**

Symbol	Description	Consensus Sequence <sup>a</sup>
PKA	cAMP-dependent	-R-R-X-S/T-Z- ( <u>SEQ ID NO: 1</u> )
PhK	phosphorylase kinase	-R-X-X-S/T-F-F- ( <u>SEQ ID NO: 2</u> )
cdk2	cyclin-dependent kinase-2	-S/T-P-X-R/K ( <u>SEQ ID NO: 3</u> )
ERK2	extracellular-regulated kinase-2	-P-X-S/T-P- ( <u>SEQ ID NO: 4</u> )
PKC	protein kinase C	KKKKRFSFK <sup>b</sup> ( <u>SEQ ID NO: 5</u> ) XRXXSXRX ( <u>SEQ ID NO: 6</u> )

**TABLE 1**

<b>Symbol</b>	<b>Description</b>	<b>Consensus Sequence<sup>a</sup></b>
CaMKI	Ca <sup>2+</sup> /calmodulin-dependent protein kinase I	RRRLSDSNF <sup>c</sup> ( <b>SEQ ID NO: 7</b> )
CaMKII	Ca <sup>2+</sup> /calmodulin-dependent protein kinase II	KKLNRTLTVA <sup>d</sup> ( <b>SEQ ID NO: 8</b> )
c-Src	cellular form of Rous sarcoma virus transforming agent	-E-E-I-Y-E/G-X-F- ( <b>SEQ ID NO: 9</b> )
v-Fps	transforming agent of Fujinami sarcoma virus	-E-I-Y-E-X-I/V- ( <b>SEQ ID NO: 10</b> )
Csk	C-terminal Src kinase	-I-Y-M-F-F-F- ( <b>SEQ ID NO: 11</b> )
InRK	Insulin receptor kinase	-Y-M-M-M- ( <b>SEQ ID NO: 12</b> )
EGFR	EGF receptor	-E-E-E-Y-F- ( <b>SEQ ID NO: 13</b> )

<sup>a</sup>see, for example, B.E. Kemp and R.B. Pearson, Trends Biochem. Sci. 15:342-346 (1990); Z. Songyang et al., Current Biology 4:973-982 (1994); J.A. Adams, Chem Rev. 101:2272 (2001) and references cited therein; X means any amino acid residue, "/" indicates alternate residues; and Z is a hydrophobic amino acid, such as valine, leucine or isoleucine

<sup>b</sup>Graff et al., J. Biol. Chem. 266:14390-14398 (1991)

<sup>c</sup>Lee et al., Proc. Natl. Acad. Sci. 91:6413-6417 (1994)

<sup>d</sup>Stokoe et al., Biochem. J. 296:843-849 (1993)

Please delete paragraph [0137] and replace it with the following paragraph:

[0137] Scheme 1 illustrates an exemplary substrate (compound 1) that can be used to detect protein kinase A. The structure of compound 1 can be represented as X-L-Dye-Ser(OPO<sub>3</sub><sup>2-</sup>)LeuArgArgArgArgPheSerLys(ε-N-Ac)Gly(NH<sub>2</sub>) (**Peptide disclosed as SEQ ID NO: 21**), wherein X is a C-16 fatty acid acyl group (palmitoyl), L is a linker (para-NHCH<sub>2</sub>C<sub>6</sub>H<sub>4</sub>C(=O)NHCH<sub>2</sub>) that links X to Dye, Dye is a fluorescent moiety (in this case, fluorescein), ε-N-Ac is an acetyl group, Ser, Leu, Arg, Phe, Ser, Lys, and Gly are standard 3-

letter codes for serine, leucine, arginine, phenylalanine, lysine, and glycine, respectively, and NH<sub>2</sub> indicates that the carboxyl group of the C-terminal glycine is an amide. An exemplary synthesis of compound 1 is described in Example 1A.

Please delete paragraph [0143] and replace it with the following paragraph:

**[0143]** Scheme 2 shows an exemplary substrate (compound 2) for detecting alkaline phosphatase activity. The compound can be represented as X-LeuArgArgArgArgPheSer (OPO<sub>3</sub><sup>2-</sup>)Lys(ε-N-Dye)Gly-NH<sub>2</sub> (**Peptide disclosed as SEQ ID NO: 22**), wherein X is a C-16 fatty acid acyl group (palmitoyl), Dye is a fluorescent moiety (fluorescein) that is linked to the epsilon amino group of a lysine residue, and NH<sub>2</sub> indicates that the carboxyl group of the C-terminal glycine is an amide. An exemplary synthesis of compound 2 is described in Example 2A. In this structure, the hydrophobic X group is linked directly by an amide bond to the N-terminal amino group of the polypeptide segment, without using additional linker atoms. However, it will be appreciated that a linker containing one or more linking chain atoms could also be included if desired.

Please delete paragraph [0147] and replace it with the following paragraph:

**[0147]** Yet another design for enzyme substrates in accordance with the invention is illustrated in Scheme 3 (see compounds 3 to 6 and 3P to 6P). Scheme 3 shows a group of compounds having different length alkyl acyl groups (X), as possible substrates for detecting a protein kinase A by fluorescence detection. The general structure of these substrates can be represented by X-Y(Dye)-LeuArgArgAlaSer(OR)LeuGly-NH<sub>2</sub> (**Peptide disclosed as SEQ ID NO: 23**), wherein X is a fatty acid acyl group of the form CH<sub>3</sub>(CH<sub>2</sub>)<sub>x</sub>C(=O)-, with x as defined in Table 1, Y is 2-aminomethylglycine, Dye is a 4,7-dichlorofluorescein dye attached to the 2-amino group of Y by a 5-carbonyl linkage to the pendant phenyl ring of the dye, R is H or PO<sub>3</sub><sup>2-</sup> (see table 1), and NH<sub>2</sub> indicates that the carboxyl group of the C-terminal glycine is an amide. An exemplary synthesis of compound 3 is shown in Example 3A.

Please delete paragraph [0153] and replace it with the following paragraph:

**[0153]** Another embodiment for enzyme substrates in accordance with the invention includes substrates wherein the hydrophobic moiety may be substituted by at least one halogen atom (e.g. fluorine). Examples of such enzyme substrates are shown in Scheme 4 and Scheme 5. Scheme 4 shows an exemplary substrate (compound 7) for detecting protein kinase A activity. The compound can be represented as X-Lys(ε-N-Dye)LeuArgArgAlaSerLeuGly-NH<sub>2</sub> (**Peptide disclosed as SEQ ID NO: 24**), wherein X is a n-(1H, 1H, 2H, 2H perfluorodecyl-1-thiol-2-acetyl group, Dye is a fluorescent moiety (5-carboxysulfofluorescein) that is linked to the epsilon amino group of a lysine residue, and NH<sub>2</sub> indicates that the carboxyl group of the C-terminal glycine is an amide. An exemplary synthesis of compound 7 is described in Example 4A. In this structure, the hydrophobic X group is linked by a thiol-2-acetyl group to the N-terminal amino group of the polypeptide segment. However, it will be appreciated that alternative linkers could also be included if desired.

Please delete paragraph [0156] and replace it with the following paragraph:

**[0156]** Scheme 5 shows another exemplary substrate (compound 8) for detecting protein kinase A activity. The compound can be represented as Dye-Lys(ε-N-X)LeuArg-ArgAlaSerLeuGly-NH<sub>2</sub> (**Peptide disclosed as SEQ ID NO: 42**), wherein X is a N-perfluoroctanoylproline that is linked to the epsilon amino group of a lysine residue, Dye is a fluorescent moiety (5-carboxy-2',7'-dipyridyl-sulfofluorescein), and NH<sub>2</sub> indicates that the carboxyl group of the C-terminal glycine is an amide. An exemplary synthesis of compound 8 is described in Example 5A. In this structure, the hydrophobic X group is linked by a proline to the epsilon amino group of a lysine residue. It will be appreciated that alternative linkers may also be included if desired. Furthermore, the fluorescent dye is linked directly by an amide bond to the N-terminal amino group of the polypeptide segment, without using additional linker atoms. However, it will be appreciated that a linker containing one or more linking chain atoms could also be included if desired.

Please delete paragraph [0159] and replace it with the following paragraph:

[0159] In still another embodiment, the present invention encompasses enzyme substrates that include a further spacer. Scheme 6 shows an exemplary substrate (compound 9) for detecting protein kinase A activity that is contemplated by this embodiment. The compound can be represented as N-Ac-ArgGlyArgProArgThrSerSerPheAlaGluGly-OOOLys( $\epsilon$ -N-Dye)Lys( $\epsilon$ -N-X)-NH<sub>2</sub> (**Peptide disclosed as SEQ ID NO: 25**), wherein X is an octadecanoyl group that is linked to the epsilon amino group of a lysine residue, Dye is a fluorescent moiety (5-carboxy-sulfofluorescein) that is linked to the epsilon amino group of a lysine residue, O is a linker provided from a 2-aminoethoxy-2-ethoxyacetyl group, and NH<sub>2</sub> indicates that the carboxyl group of the C-terminal glycine is an amide. An exemplary synthesis of compound 9 is described in Example 6A. In this structure, the hydrophobic X group is linked to the epsilon amino group of a lysine residue without any further linker atoms. However, it will be appreciated that a linker containing one or more linking chain atoms could also be included if desired. Furthermore, the fluorescent dye is linked directly by an amide bond to the epsilon amino group of a lysine residue, without using additional linker atoms. However, it will be appreciated that a linker containing one or more linking chain atoms could also be included if desired.

Please delete paragraph [0162] and replace it with the following paragraph:

[0162] Further examples of kinase substrates wherein a linker is incorporated are shown in Table 3.

Table 3

Kinase	Peptide	RFUs at 10 uL (initial → final)	Conc (uM)	Fold increase
PKA	C13-K(dye2)-LRRASLG-NH <sub>2</sub> ( <b>SEQ ID NO: 26</b> )	1000 → 5000	8	5x
PKA	C13-OOOK(dye2)-LRRASLG-NH <sub>2</sub> ( <b>SEQ ID NO: 26</b> )	1000 → 5000	8	5x
PKC	C16-OOOK(dye2)-RREGSFR-NH <sub>2</sub> ( <b>SEQ ID NO: 27</b> )	650 → 3000	4	4.5x
PKC	C17-OOOK(tet)-RQGSFRA-NH <sub>2</sub> ( <b>SEQ ID NO: 28</b> )	700 → 4900	6	7x
Src, lyn, fyn	C16-OOOK(dye2)RIGEGTYGVVRR-NH <sub>2</sub> ( <b>SEQ ID NO: 29</b> )	1000 → 6500	8	6.5x
Akt	C15-OOOK(dye2)RPRTSSF-NH <sub>2</sub> ( <b>SEQ ID NO: 30</b> )	1500 → 7500	8	4x
MAPK	C17-OOOK(dye2)PRTPGGR-NH <sub>2</sub> ( <b>SEQ ID NO: 31</b> )	1100 → 5700	16	5x
MAPKAP2	C16-OOOK(dye2)RLNRTLSV-NH <sub>2</sub> ( <b>SEQ ID NO: 32</b> )	800 → 3200	8	4x

Please delete paragraph [0165] and replace it with the following paragraph:

[0165] The compound can be represented as N-X-OOOLys(ε-N-Dye)ArgArgGluGly-SerPheArg-NH<sub>2</sub> (**Peptide disclosed as SEQ ID NO: 27**), wherein X is an hexadecanoyl group that is linked to the □-amino group of the lysine residue by the linker -OOO-, Dye is a fluorescent moiety (5-carboxy-2',7'-dipyridyl-sulfofluorescein) that is linked to the epsilon amino group of the lysine residue, O is a linker provided from a 2-aminoethoxy-2-ethoxyacetyl group, and NH<sub>2</sub> indicates that the carboxyl group of the C-terminal glycine is an

amide. An exemplary synthesis of compound 10 is described in Example 7A. In this structure, the hydrophobic X group is linked to the  $\square$ -amino group of the lysine residue by the linker -OOO-. It will be appreciated that alternative linkers could also be included if desired. Furthermore, the fluorescent dye is linked directly by an amide bond to the N-terminal amino group of the polypeptide segment, without using additional linker atoms. However, it will be appreciated that a linker containing one or more linking chain atoms could also be included if desired.

Please delete paragraph [0180] and replace it with the following paragraph:

[0180] Example 8 describes an inhibition study with a protein kinase A (see also FIG. 4). PKA from E. coli was incubated in the presence of different concentrations of ATP (50, 10, 3 and 2  $\mu$ M adenosine triphosphate) in the absence (lowest trace) or presence of the inhibitor staurosporine (5 nM, middle trace) or the PKA-specific inhibitor TYADFIASGRTGRRNAI (**SEQ ID NO: 20**) (20 nM, highest trace). The fluorescent substrate for phosphorylation had the structure: N-palmitoyl-alpha-2-aminomethyl-Gly(5-carboxy-sulfonefluorescein)LeuArgArgAlaSer(OH)LeuGly-NH<sub>2</sub> (**Peptide disclosed as SEQ ID NO: 33**) (compound 11), wherein a hydrophobic moiety (palmitoyl) and a fluorescent moiety (Dye) are both linked to the N-terminal residue of the kinase recognition moiety, similar to structure shown in Scheme 3 above. The Dye, 5-carboxy-sulfonefluorescein, is linked to the N-terminal residue by an amide bond formed between the 5-carbonyl group and the 2-amino nitrogen of the 2-aminomethyl group. The palmitoyl group is coupled to the N-terminal residue via the alpha amino nitrogen. The structure is shown in Scheme 8, and a synthetic procedure is provided in Example 8A.

Please delete paragraph [0198] and replace it with the following paragraph:

[0198] An exemplary enzyme substrate useful for detecting protein kinase A, palmitoyl-FAM-S(OPO<sub>3</sub><sup>2-</sup>)LRRRRFSK(Ac)G-amide (Peptide disclosed as SEQ ID NO: 43), was prepared as follows. The peptide Fmoc-L(R(Pmc))<sub>4</sub>FS(tBu)K(Mt)G (Peptide disclosed as SEQ ID NO: 44) was constructed via solid phase peptide synthesis using standard FastMoc™ chemistry on 625 mg of Fmoc-PAL-PEG-PS resin at 0.16 mmol/g, a solid support which results in a carboxamide peptide. A portion of the final protected peptide-resin (20 mg, 2 µmol peptide) was transferred to a 1.5 ml eppendorf tube and treated with 1 mL of 5% trifluoroacetic acid (TFA) in dichloromethane (DCM), giving a characteristic yellow trityl color. The resin was precipitated by the addition of 0.1 mL methanol and washed (3 x 1 mL dimethylformamide, DMF). Diisopropylethylamine (50 µL) and capping solution (1 mL of a solution of acetic anhydride (0.5 M) and hydroxybenzotriazole (0.015 M) in N-methylpyrrolidone (NMP)) were added to the resin and the mixture was agitated for 10 minutes. The resin was washed (3 x 1 mL DMF) and treated with piperidine (1 mL of 20% piperidine in DMF). After 4 minutes, the resin was washed with DMF (6 x 1 mL). The resin was treated with Fmoc-Ser(OPO(OBzl)OH)-OH (10 mg), coupling solution (50 µL of a solution of HBTU (2-(1H-benzotriazol-1-yl)1,1,3,3-tetramethyluronium hexafluorophosphate, 0.45 M) and HOBT (1-hydroxybenzotriazole, 0.45 M) and diisopropylethylamine (20 µL). After agitation for 35 minutes, the resin was washed with DMF (3 x 1 mL) and treated with piperidine (1 mL of 20% piperidine in DMF). After 5 minutes the resin was washed with DMF (6 x 1 mL) and treated with 4'-(para-(Fmoc-NHCH<sub>2</sub>)C<sub>6</sub>H<sub>4</sub>C(=O)NHCH<sub>2</sub>)-5- FAM succinimidyl ester (10 mg) and diisopropylethylamine (35 uL). After 1 h of agitation the resin was washed (6 x 1 mL DMF) and treated with piperidine (20% piperidine in NMP). After 5 minutes the resin was washed with NMP (6 x 1 mL) and treated with palmitoyl chloride (5 µL) and diisopropylethylamine (35 µL). After 16 minutes of agitation the resin was washed (3 x 1 mL NMP, 1x1 mL 1:1 methanol/DCM), and dried in a vacuum centrifuge. The peptide was cleaved from the resin with 1 mL cleavage solution (950 µL TFA, 50 µL water, 25 µL triisopropylsilane, and 25 µL thioanisole). After 1.5 to 2 h the mixture was filtered and the filtrate concentrated to dryness on a rotary evaporator. The residue was

dissolved in water (0.5 mL) and a portion purified by reverse-phase HPLC (Metachem Technologies column: 150x4.6 mm, Polaris C18, 5  $\mu$ m) using a 10% to 40% gradient over 10 min of 0.1% TFA in acetonitrile vs. 0.1% TFA in water. The dye-labeled peptide was analyzed by ESI mass spectrometry, which resulted in the expected M/z = 2141.

Please delete paragraph [0200] and replace it with the following paragraph:

[0200] Synthesis of an exemplary dye-labeled peptide, compound 2, palmitoyl-LRRRRFS(OPO<sub>3</sub><sup>2-</sup>)K(5-FAM)G-amide (SEQ ID NO: 34), is described below. The peptide Fmoc-LR(Pmc)<sub>4</sub>FS(OPO(OBzl)OH)K(Mtt)G (Peptide disclosed as SEQ ID NO: 35) was constructed via solid phase peptide synthesis using standard FastMoc™ chemistry on 625 mg of Fmoc-PAL-PEG-PS resin at 0.16 mmol/g, a solid support which results in a carboxamide peptide. Peptides with a carboxy terminus were constructed using Fmoc-Gly-PEG-PS resin. A portion of the final protected peptide-resin (20 mg, 2  $\mu$ mol peptide) was transferred to a 1.5 ml eppendorf tube and treated with 1 mL of 5% trifluoroacetic acid (TFA) in dichloromethane (DCM), giving a characteristic yellow trityl color. The resin was precipitated by the addition of 0.1 mL methanol and washed (3 x 1 mL dimethylformamide, DMF). 5-Carboxyfluorescein succinimidyl ester (5 mg, 10  $\mu$ mol), diisopropylethylamine (30  $\mu$ L, 173  $\mu$ mol) and DMF (100  $\mu$ L) were added to the resin and the mixture was agitated gently for 2-10 h. The resin was washed (5 x 1 mL DMF), treated 5 minutes with piperidine (1 mL of 20% piperidine in NMP). The resin was washed with NMP (3 x 1 mL NMP). Palmitoyl chloride (5  $\mu$ L) and diisopropylethylamine (35  $\mu$ L) were added to the resin and the mixture agitated for 10 minutes. The resin was washed (3 x 1 mL NMP, 1x1 mL 1:1 methanol/DCM), and dried in a vacuum centrifuge. The peptide was cleaved from the resin with 1 mL cleavage solution (950  $\mu$ L TFA, 50  $\mu$ L water, 25  $\mu$ L triisopropylsilane, and 25  $\mu$ L thioanisole). After 1.5 to 2 h the mixture was filtered and the filtrate concentrated to dryness on a rotary evaporator. The residue was dissolved in water (0.5 mL) and a portion purified by reverse-phase HPLC (Metachem Technologies column: 150x4.6 mm, Polaris C18, 5  $\mu$ m) using a 10% to 40% gradient over 10 min of 0.1% TFA in acetonitrile vs. 0.1% TFA in water.

The dye-labeled peptide was analyzed by ESI mass spectrometry, which resulted in the expected M/z = 1850.

Please delete paragraph [0203] and replace it with the following paragraph:

[0203] A series of compounds (Scheme 3 above) having different length alkylacyl groups were prepared in both phosphorylated and unphosphorylated form, represented by the following formula: X-Y(Dye)LRRAS(OR)LG-NH<sub>2</sub> (**Peptide disclosed as SEQ ID NO: 23**), wherein X is a fatty acid acyl group of the form CH<sub>3</sub>(CH<sub>2</sub>)<sub>x</sub>C(=O)-, x is 0, 7, 10, or 14, Y is alpha-aminomethyl glycine, Dye is a 4,7-dichlorofluorescein dye attached to the 2-amino nitrogen atom of Y by a 5-carbonyl linkage to the pendant phenyl ring of the dye, and R is H or PO<sub>3</sub><sup>2-</sup>.

Please delete paragraph [0204] and replace it with the following paragraph:

[0204] For compounds 3-6, the peptide Fmoc-L(R(Pmc))<sub>2</sub>AS(tBu)LG (**Peptide disclosed as SEQ ID NO: 36**) was constructed via solid phase peptide synthesis using standard FastMoc™ chemistry on 625 mg of Fmoc-PAL-PEG-PS resin at 0.16 mmol/g, a solid support which results in a carboxamide peptide. A representative synthesis of compound 4 follows.

Please delete paragraph [0206] and replace it with the following paragraph:

[0206] Compounds 3, 5, and 6 were prepared in the same manner, except substituting nonanyl chloride with either acetic anhydride (compound 3), lauryl chloride (compound 5) or palmitoyl chloride (compound 6). Compounds 3P, 4P, 5P and 6P were made similarly to compounds 3-6, except that the peptide fmocLR(Pmc)<sub>2</sub>AS(OPO(OBzl)OH)LG (**SEQ ID NO: 37**) on PAL resin was used.

Please delete paragraph [0208] and replace it with the following paragraph:

**[0208]** The synthesis of an exemplary dye-labeled peptide, compound **7**, N-(1H,1H,2H,2H-Perfluorodecyl-1-thiol-2-acetyl)-K-(5-carboxysulfofluorescein) LRRASL-G-amide (Peptide disclosed as SEQ ID NO: 26), is described below. The peptide Fmoc-K(ivDde)LRRASLG (Peptide disclosed as SEQ ID NO: 26) was constructed via solid phase peptide synthesis using standard FastMoc™ chemistry on 625 mg of Fmoc-PAL-PEG-PS resin at 0.16 mmol/g, a solid support which results in a carboxamide peptide. A portion of the final protected peptide-resin (20 mg, 2 µmol peptide) was transferred to a 1.5 mL Eppendorf tube and treated with 20% piperidine (500 µL) in dimethylformamide (DMF) for 20 minutes to remove the FMOC protecting group. The resin was then washed with 3 x 1 mL of DMF followed by 3 x 1 ml of methylene chloride (DCM). Iodoacetic acid (2.5 mg, 13 µmol), 0.2 M Dicyclohexylcarbodiimide in ethyl acetate (EtOAc) (70 µl, 13 µmol), 0.2 M N-hydroxysuccinimide in EtOAc (200 µL, 40µmol) and DMF (100µL) were combined and allowed to stand for 30 minutes. This mixture was added to the resin and was agitated gently for 3 h. The resin was washed (5 x 1 mL DMF) followed by 5 x 1 ml DCM. 1H,1H,2H,2H Perfluorodecyl-1-thiol (35 mg, 75 µmol) in 100 µL DMF was added to the resin and was agitated gently for 15 hrs. The resin was washed (5 x 1 mL DMF) followed by 5 x 1 ml DCM. The ivDde protecting group was removed by treating the resin with 10% hydrazine (500 µL) in DMF for 20 minutes. The resin was washed with 3 x 1 mL DMF followed by 3 x 1 ml DCM. 5-carboxysulfofluorescein (5 mg, 10 µmol), 0.45M HOBT/HBTU (40 µl, 18 µmol), 2 M diisopropylethylamine in NMP (20 µL, 40 µmol) and DMF (100 µL) were added to the resin and the mixture was agitated gently for 3 h. The resin was washed (5 x 1 mL DMF) followed by 5 x 1 ml DCM. The peptide was cleaved from the resin with 1 mL cleavage solution (950 µL TFA, 50 µL water, 25 µL triisopropylsilane, and 25 µL thioanisole). After 1.5 to 2 h the mixture was filtered and the filtrate concentrated to dryness on a rotary evaporator. The residue was triturated with 3 x 1mL hexane washes and a portion purified by reverse-phase HPLC (Agilent column: 150x4.6 mm, 300 Extend, 5 µM) using a 25% to 70% gradient over 25 min of 0.1% TFA in acetonitrile vs. 0.1% TFA in water. The dye-labeled peptide was analyzed by ESI mass spectrometry, which resulted in the expected M/z = 1814.

Please delete paragraph [0210] and replace it with the following paragraph:

[0210] The synthesis of an exemplary kinase substrate, compound **8**, (5-carboxy-2,7'-dipyridyl-sulfofluorescein)-K-(N-perfluoro-octanoyl-proline)-LRRASLG-amide (Peptide disclosed as SEQ ID NO: 26), follows. The peptide Fmoc-K(ivDde)LRRASLG (Peptide disclosed as SEQ ID NO: 26) was constructed via solid phase peptide synthesis using standard FastMoc™ chemistry on 625 mg of Fmoc-PAL-PEG-PS resin at 0.16 mmol/g, a solid support which results in a carboxamide peptide. A portion of the final protected peptide-resin (20 mg, 2 µmol peptide) was transferred to a 1.5 mL Eppendorf tube and treated with 20% piperidine (500µL) in dimethylformamide (DMF) for 20 minutes to remove the FMOC protecting group. The resin was then washed with 3 x 1 mL of DMF followed by 3 x 1 mL of methylene chloride (DCM). 5-carboxy-2,7'-dipyridyl-sulfofluorescein (5 mg, 9 µmol), 0.45M HOBT/HBTU (40 µL, 18 µmol), 2M diisopropylethylamine in NMP((20 µl, 40 µmol) and DMF (100µL) were added to the resin and the mixture was agitated gently for 3hrs. The resin was washed (5 x 1 mL DMF) followed by 5 x 1 mL DCM. The ivDde protecting group was removed by treating the resin with 10% hydrazine (500 µL) in DMF for 20 minutes. The resin was washed with 3 x 1 mL DMF followed by 3 x 1 mL DCM. (N-Perfluorooctanoyl L-proline (prepared by the method of Curran and Luo, JACS 1999, 121, 9069-9072; 25 mg, 49 µmol), 0.45M HOBT/HBTU (40 µL, 18 µmol) and 2M DIPEA/NMP (20 µl, 40 µmol) were added to the resin and agitated gently for 15hrs. The resin was washed (5 x 1 mL DMF) followed by 5 x 1 ml DCM. The peptide was cleaved from the resin with 1 mL cleavage solution (950 µL TFA, 50 µL water, 25 µL triisopropylsilane, and 25 µL thioanisole). After 1.5 to 2 h the mixture was filtered and the filtrate concentrated to dryness on a rotary evaporator. The residue was triturated with 3 x 1 mL hexane washes and a portion purified by reverse-phase HPLC (Agilent column: 150 x 4.6 mm, 300 EXTEND, 5 µm) using a 25% to 70% gradient over 25 min of 0.1% TFA in acetonitrile vs. 0.1% TFA in water. The dye-labeled peptide was analyzed by ESI mass spectrometry, which resulted in the expected M/z = 1940.

Please delete paragraph [0212] and replace it with the following paragraph:

[0212] The synthesis of exemplary substrate, compound **9**, N-AcetylRGRPRTSSFAEG-OOK(N-5-Carboxysulfo-fluroescein)K(N-Octadecanoyl)-amide (Peptide disclosed as SEQ ID NO: 25), where O is a linker provided from a 2-aminoethoxy-2-ethoxyacetyl group, is described below. The peptide N-AcetylRGRPRTSSFAEG(AEEA)<sub>3</sub>K(ivDde)K(Mtt) (Peptide disclosed as SEQ ID NO: 38) was constructed via solid phase peptide synthesis using standard FastMoc™ chemistry on 625 mg of Fmoc-PAL-PEG-PS resin at 0.16 mmol/g, a solid support which results in a carboxamide peptide. A portion of the final protected peptide-resin (20 mg, 2 µmol peptide) was transferred to a 1.5 ml Eppendorf tube and treated with 5% trifluoroacetic acid (TFA) in dimethylformamide (DMF) (4 x 200 µL with 5 minute wait per treatment) to remove the mtt protecting group. The resin was then washed with 3 x 1 mL of DMF followed by 3 x 1 mL of methylene chloride (DCM) and is treated with octadecanoic acid (25 mg, 88 µmol), 0.45 M HOBT/HBTU(100 µl, 45 µmol), 2 M DIPEA in NMP (40 µL, 80 µmol) and DMF (200 µL). The mixture was gently agitated for 2 hrs and the resin was washed with 3 x 1 mL of DMF followed by 3 x 1 mL of DCM. The resin was treated with 10% hydrazine in DMF (500 µL) for 20 minutes to remove the ivDde protecting group, followed by washing with 3 x 1 mL DMF and 3 x 1 ml DCM. 5-carboxy-sulfofluorescein (5 mg, 9 µmol), 0.45 M HOBT/HBTU (40 µL, 18 µmol), 2 M DIPEA in NMP (20 µL, 40 µmol) and DMF (100 µL) were added to the resin and the mixture was agitated gently for 3 h. The resin was washed with 5 x 1 mL DMF followed by 5 x 1 mL DCM. The peptide was cleaved from the resin with 1 mL cleavage solution (950 µL TFA, 50 µL water, 25 µL triisopropylsilane, and 25 µL thioanisole). After 1.5 to 2 hrs the mixture was filtered and the filtrate concentrated to dryness on a rotary evaporator. The residue was triturated with 3 x 1 mL hexane washes and a portion purified by reverse-phase HPLC (Agilent column: 150 x 4.6 mm, 300 EXTEND, 5 µM) using a 25% to 70% gradient over 25 min of 0.1% TFA in acetonitrile vs. 0.1% TFA in water. The dye-labeled peptide was analyzed by ESI mass spectrometry, which resulted in the expected M/z = 2714.

Please delete paragraph [0214] and replace it with the following paragraph:

[0214] Synthesis of (N-palmitoyl)-Lys(N-5-carboxy-2,’7’-dipyridyl-sulfofluorescein)- OOO-RREGSFR-amide (Peptide disclosed as SEQ ID NO: 27), is described below. The abbreviation O describes a linker provided from a 2-aminoethoxy-2-ethoxyacetyl group. The peptide Fmoc-OOOK(ivDde)RREGSFR (Peptide disclosed as SEQ ID NO: 27) was constructed via solid phase peptide synthesis using standard FastMoc™ chemistry on 625 mg of Fmoc-PAL-PEG-PS resin at 0.16 mmol/g, a solid support which results in a carboxamide peptide. A portion of the final protected peptide-resin (20 mg, 2 µmol peptide) was transferred to a 1.5 mL Eppendorf tube and treated with 20% piperidine (500 µL) in dimethylformamide (DMF) for 20 minutes to remove the fmoc protecting group. The resin was washed with 3 x 1 mL DMF followed by 3 x 1 mL DCM. Palmitic acid (20 mg, 78 µmol), 0.45M HOBT/HBTU (100 µL, 45 µmol), 2M diisopropylethylamine/NMP (40 µL, 80 µmol) were added to the resin and agitated gently for 2 h followed by the above washes. The ivDde protecting group was removed by treating the resin with 10% hydrazine (500 µL) in DMF for 20 minutes. The resin was washed with 3 x 1 mL DMF followed by 3 x 1 mL DCM.

Please delete paragraph [0217] and replace it with the following paragraph:

[0217] A fluorescent PKA substrate (compound 11) having the structure, N-palmitoyl-alpha-(2-aminomethyl)glycine(5-carboxy-sulfonefluorescein)LeuArgArgAlaSer(OH)Leu-Gly-NH<sub>2</sub> (Peptide disclosed as SEQ ID NO: 33), was prepared by a method similar to that used to make compound 6 above, but with the following changes. Instead of palmitoyl chloride, palmitic acid (5 mg), coupling solution (100 µL) and diisopropylethylamine (30 µL) were used for addition of the hydrophobic moiety. Also, the reaction to attach the fluorescent dye involved the use of 5-carboxysulfonefluorescein (5 mg), coupling solution (50 µL) and diisopropylamine (20 µL). This resulted in attachment of the fluorescent dye to the alpha-amino group of the N-terminal alpha-(2-aminomethyl)glycine residue, and attachment of the 5-carboxy-sulfonefluorescein to 2-amino nitrogen of the 2-aminomethyl group of the same residue by amide linkage to the 5-carbonyl group of the dye.

Please delete paragraph [0218] and replace it with the following paragraph:

[0218] Reaction mixtures (100  $\mu$ L) were prepared containing 20 mM Tris-HCl, pH 8.1, 1 mM MgCl<sub>2</sub>, 1  $\mu$ M compound, and 3 units of protein kinase A. Reactions were initiated by addition of ATP to a final concentration of 50, 10, 3 and 2  $\mu$ M. Fluorescence data were collected on a Perkin-Elmer LS-50B luminescence spectrometer at an excitation of 480 nm and emission of 520 nm. The assay was repeated in the presence of staurosporine (5 nM) or a PKA-specific peptide inhibitor (20 nM) TYADFIASGRTGRRNAI (Peptide disclosed as SEQ ID NO: 20). Results are shown in Fig. 4.

Please delete paragraph [0219] and replace it with the following paragraph:

[0219] A PKC substrate was prepared having the following structure (compound 12): alpha-palmitoyl-Lys( $\epsilon$ -N-5-carboxy-sulfonefluorescein)S(OPO<sub>3</sub><sup>2-</sup>)KLKRQGSFKY-amide (Peptide disclosed as SEQ ID NO: 39), wherein a palmitoyl group is linked to the alpha amino group of the N-terminal lysine residue by an amide linkage, and the fluorescein dye was linked to the epsilon nitrogen atom of the same lysine residue by an amide linkage to the 5-carboxy group of the dye. The synthetic procedure was similar for that of the PKA substrates described above, except that Fmoc-S(PO(OBzl)OH)KLKRQGSFKY (Peptide disclosed as SEQ ID NO: 40) was formed on PAL resin, and Fmoc-Dpr(ivDde) was replaced with Fmoc-Lys(ivDde).

Please delete paragraph [0221] and replace it with the following paragraph:

[0221] A substrate (compound 13) was prepared having the structure: N-palmitoyl-Lys( $\epsilon$ -N-5-carboxy-sulfonefluorescein)KVEKIGEGTYGVVKK-amide (Peptide disclosed as SEQ ID NO: 41). The synthetic protocol was similar to that used for synthesis of compound 8, except that a peptide-resin Fmoc-Lys(ivDde)-KVEKIGEGTYGVVKK (Peptide disclosed as SEQ ID NO: 41) was used. Results are shown in Fig. 6.